Docket No.: 30694/41506
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of:
Kollet et al.

Application No.: 10/552,299 | Confirmation No.: 2069

Filed: August 25, 2006 | Art Unit: 1632

For: STEM CELLS HAVING INCREASED SENSITIVITY TO SDF-1 AND METHODS OF GENERATING AND USING SAME | Examiner: Shen, Wu Cheng Winston

**SUBMISSION FOR PRE-APPEAL CONFERENCE**

MS AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicants request review of the rejection under 35 U.S.C. § 103(a) contained in the final Office Action mailed January 20, 2010 ("final Office Action"), in the above-identified matter. Each of the claims under examination, *i.e.,* claims 30-36, 38, and 39, has been rejected twice and is now finally rejected. Therefore, claims 30-36, 38, and 39 are in condition for the requested review. No amendments are being filed with this request. This paper is filed concurrently with a Notice of Appeal and fee, and includes no more than five pages of patentability argument.

## I. The Invention

The invention relates to methods of generating stem cells suitable for transplantation comprising collecting stem cells, exposing the stem cells to an exogenous matrix metalloprotease or an active portion thereof, and isolating stem cells having increased CXCR4 levels compared to stem cells not exposed to the matrix metalloprotease or an active portion thereof, to thereby generate stem cells suitable for transplantation (claim 30).

## II. The Pending Rejection Should Be Withdrawn

Claims 30-36, 38 and 39 stand rejected under 35 U.S.C. § 103(a) for assertedly being obvious in view of Kollet et al., Blood, 97(10):3283-91 (2001) (hereinafter "Kollet"), Heissig et al., Cell, 109(5):625-37 (2002) (hereinafter "Heissig"), Rafii et al., U.S. Patent Publication No. 2004/0071687 (hereinafter "Rafii"), Togawa et al., Cancer Lett., 146(1):25-33 (1999) (hereinafter "Togawa"), and Sadatmansoori et al., Protein Expr. Purif., 23(3):447-52 (2001) (hereinafter "Sadatmansoori"). The Examiner maintained the rejection in an Advisory Action mailed June 1, 2010. The rejection, however, lacks the support required to establish a *prima facie* basis for obviousness. Accordingly, Applicants request that the rejection be reversed.

### *A. The Examiner relied on two errors of fact.*

#### 1. Kollet et al. does not teach that increased SDF-1 levels lead to up-regulation of CXCR4, as asserted by the Examiner.

To establish a *prima facie* case of obviousness, all claimed elements must be disclosed or suggested in the prior art and a reason to combine the teachings of multiple references, or to modify the teaching of a single reference, must exist. See *KSR International Co. v. Teleflex Inc. et al.*, 550 U.S. 398, 498, 127 S.Ct. 1727, 1731 (2007). Moreover, any proposed modification or combination of the prior art must be supported by a reasonable expectation of success, determined from the vantage point of the skilled artisan at the time the invention was made. *Amgen Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1208-09 (Fed. Cir. 1991). The suggestion or motivation to make the invention and the reasonable expectation of success must be derived from the prior art, and not from Applicant's disclosure. See M.P.E.P. §§ 2142-43.

The basis of the Examiner's rejection of the claims is that Kollet teaches that increased stromal derived factor-1 (SDF-1) levels lead to up-regulation of CXCR4, that Heissig teaches that increased levels of SDF-1 lead to up-regulation of the matrix metalloprotease MMP-9, and that Rafii teaches that MMP-9 promotes release of SDF-1. Thus, according to the Examiner, because MMP-9 promotes release of SDF-1 and SDF-1 leads to up-regulation of both MMP-9 and CXCR4, one of skill in the art would expect that administration of MMP-9 would lead to up-regulation of SDF-1, in turn leading to up-regulation of CXCR4. The Examiner, however, has misconstrued the disclosures in the cited references.

Support for the Examiner's rejection is based on an error of fact in that the Examiner asserted that "pretreatment of cells with cytokines (e.g. SDF-1) lead to up-regulation of CXCR4

expression, which increased both *in vitro* migration to SDF-1 and *in vivo* homing." See final Office Action, p. 8. Kollet, however, disclosed that pretreatment of the cells *in vitro* with SCF (not SDF-1) and IL-6 induced increased surface expression of CXCR4 and migration toward SDF-1. Moreover, the pretreatment with SCF (not SDF-1) and IL-6 occurred *in vitro* and was followed by injection of the pretreated cells into mice. Kollet did not demonstrate that pretreatment of cells with SDF-1 (rather than SCF and IL-6) led to up-regulation of CXCR4, increased migration to SDF-1, and increased *in vivo* homing, as the Examiner asserted. Instead, it was pretreatment of cells with SCF and IL-6 that caused up-regulation of CXCR4 (the receptor for SDF-1), allowing increased BM homing of the stem cells to the SDF-1 gradient. The Examiner appears to have been aware that Kollet disclosed treatment of stem cells with SCF, not SDF-1, in reciting that "[i]solated $CD34^+$ cells were either used immediately for homing experiments or after overnight incubation with RPMI supplemented with 10% fetal calf serum (FCS) and stem cell factor (SCF) (50 ng/mL)." See final Office Action, pp. 6-7. Having acknowledged that Kollet's cells were pretreated with SCF, not SDF-1, it is hoped that the Examiner agrees that the assertion that Kollet pretreated cells with SDF-1 (see p. 8 of the same final Office Action) was an error of fact.

The Examiner also stated that Heissig provided the molecular mechanism (MMP-9 increasing bone marrow (BM) homing) for the teaching of Kollet, which the Examiner again characterized as the pretreatment of cells with SDF-1 leading to up-regulation of CXCR4. *Id.*, at p. 10. Kollet, however, did not teach that pretreatment of cells with SDF-1 leads to up-regulation of CXCR4 expression. Kollet et al. performed experiments to test the potential of human SDF-1 to attract stem cells *in vivo*. To that end, Kollet showed that injection of human SDF-1 into the BM of NOD/SCID mice increased the number of primitive $CD34^+CD38^{-/low}$ cells homing to the BM. See p. 3287. In the same paragraph, the authors describe pretreating $CD34^+$ or $CD34^+CD38^{-/low}$ cells with Stem Cell Factor (SCF) (not SDF-1). Thus, the Examiner erred on the facts in asserting that Heissig taught the molecular mechanism (MMP-9-mediated induction of CXCR4) as underlying Kollet's observations that pre-treatment of cells with SDF-1 leads to increased CXCR4, because Kollet made no such observation.

2. Rafii does not teach that MMP-9 promotes the release of SDF-1, as asserted by the Examiner.

The Examiner relied on a second factual error in asserting that Rafii "teaches that MMP-9 promotes release of stem cell active cytokines (e.g. SDF-1), thereby promoting expansion of quiescent stem cells, and this novel concept lays the foundation of developing strategies where

activation of proteases such as MMP-9 may act as molecular switches to expand a large population of stem cells that may ultimately be used for organ-regeneration and tissue vascularization." See final Office Action, p. 11. This represents an exact quote of paragraph 114 of Rafii except for the unsupported addition of "(e.g. SDF-1)" by the Examiner. In fact, nowhere in Rafii is there a disclosure or suggestion of SDF-1 as a stem cell-active cytokine whose release is promoted by MMP-9.

In paragraph 114, Rafii discloses that MMP-9 promotes the release of an otherwise tethered stem cell-active cytokine known as Kit-ligand, or SCF. Thus, in paragraph 114, Rafii refers to soluble Kit-ligand (sKitL) as the stem cell-active cytokine released by MMP-9, not SDF-1. Even if construed more generally, Rafii's reference to "stem cell active cytokines" embraces an unknown number of proteins without any hint or suggestion that SDF-1, specifically, is one of those proteins. Further, taken in context, the "release" of stem cell-active cytokines refers to the normally membrane-tethered Kit-ligand and its "release" from the membrane by the proteolytic action of MMP-9. See Rafii, ¶ 110. Importantly, SDF-1 does not share the membrane-tethering feature of Kit-ligand and, thus, SDF-1 cannot be "released" in the manner in which sKitL is released. Finally, Rafii explicitly states that MMP-9 functions downstream of SDF-1 (*see* Rafii, ¶ 107, explaining that MMPs are necessary intermediates downstream of SDF-1). Accordingly, MMP-9 would not be expected to affect SDF-1 activity. For each of these reasons, the Examiner erred as a matter of fact in construing Rafii as disclosing that MMP-9 promotes release of SDF-1.

The Examiner goes on to use the above-quoted language as a basis to combine Heissig and Rafii to yield a combined teaching of a "functional positive feedback loop of increased SDF-1/CXCR4 interaction and increased MMP-9 expression in regulation of hematopoietic stem cells (HSCs) mobilization and differentiation." See final Office Action, p. 11. No such positive feedback loop is disclosed or suggested by Rafii and/or Heissig because Rafii does not teach that MMP-9 promotes release of SDF-1. Both Rafii and Heissig teach that increased levels of SDF-1 lead to up-regulation of MMP-9. See Rafii, paragraph 199 and claims 1 and 9; Heissig, p. 630, left column, last paragraph. The positive feedback loop proposed by the Examiner would lead to runaway expression of SDF-1 and MMP-9. In other words, an increase in MMP-9 would lead to an increase in SDF-1, which would lead to a further increase in MMP-9, in an ever-escalating spiral of mutually increased expression. None of the references cited by the Examiner, alone or in combination, teach or suggest the positive feedback loop proposed by the Examiner. The Examiner's rebuttal to this point appears to be that the references do not disclose runaway

expression, so the SDF-1/MMP-9 interaction must be regulated, without pointing to any support for such regulation. Rather than positing unsupported regulatory schemes, the Examiner's position that MMP-9 promotes SDF-1 release should be recognized as factual error, unsupported by the disclosures of Rafii or Heissig.

Accordingly, the rationale articulated by the Examiner is flawed in that it is based on errors of fact that mischaracterize the teachings in Kollet and Rafii.

*B. The Examiner erred as a matter of law.*

The Examiner used improper hindsight reconstruction of the invention to arrive at the asserted basis of rejection. This is apparent because the references cited by the Examiner do not teach that SDF-1 increases CXCR4 levels. Thus, it was incorrect to assert that a person of skill in the art would have expected MMP-9 to lead to up-regulation of SDF-1 and, in turn, CXCR4. Moreover, the Examiner's exemplification of a stem cell-active cytokine as SDF-1, when viewed in light of Rafii's disclosure predominantly concerning soluble Kit-ligand (sKitL or SCF), can only be seen as the product of the impermissible use of hindsight reconstruction of the claims, an error of law.

## III. Conclusion

None of the legal or factual errors addressed above is remedied by the cited references, considered alone or in any combination, and the Examiner has not contended otherwise. Accordingly, the rejection of claims 30-36, 38, and 39 as obvious under 35 U.S.C. § 103(a) over Kollet in view of Heissig, Rafii, Togawa, and Sadatmansoori should be reversed.

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Respectfully submitted,

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